Data Evaluation Record on the aerobic biotransformation of cetyl pyridinium chloride monohydrate in soil

PMRA Submission Number {.....} EPA MRID Number 48012105

Data Requirement: PMRA Data Code:
EPA DP Barcode: 376391
OECD Data Point: OECD 307
EPA Guideline: 835.4100

Test material:
Common name: Cetyl pyridinium chloride.
Chemical name:
IUPAC name: 1-Hexadecylpyridinium chloride.
CAS name: Not reported.
CAS No: 6004-24-6 for monohydrate (123-03-5 for anhydrous).
Synonyms: Cetylpyridinium chloride.
SMILES string:

Primary Reviewer: James Breithaupt, Agronomist
Signature:
Antimicrobial Division (AD)
Date: 6/30/10

Risk Assessment and Science Support Branch (RASSB)
James Breithaupt
6/30/10

Final Reviewer: Nader ElkassabanyChief
Signature: Nader Elkassabany
Antimicrobial Division (AD)
Date: 6/30/10

Risk Assessment and Science Support Branch (RASSB)

Company Code:
Active Code:
Use Site Category:
EPA PC Code: 069160.

CITATION: Tan, I. N. 2009. Determination of the aerobic degradation route of ^{14}C-labelled cetylpyridinium chloride in soil. Unpublished study performed by NOTOX B.V., 's-Hertogenbosch, The Netherlands; sponsored and submitted by Vertellus Health and Specialty Products LLC, Zeeland, Michigan (p. 1). NOTOX Project No.: 487900. Experimental start date March 9, 2009 (date of application), and completion date October 28, 2009 (pp. 7, 12). Final report issued December 7, 2009.

Data Evaluation Record on the aerobic biotransformation of cetyl pyridinium chloride monohydrate in soil

PMRA Submission Number {.....} EPA MRID Number 48012105

EXECUTIVE SUMMARY

Study Acceptability: This study is classified as acceptable and satisfies the 835.4100/OECD 307 data requirement. There were some guideline deviations, but they do not invalidate the study. The DT50 was 10 days, and the DT90 was 33 days. Dissipation of cetyl pyridinium chloride (monohydrate) occurred through formation of one major aqueous soluble product, Metabolite II (m/z 194.117), several minor products, bound residues and extensive mineralization to CO_2.

The biotransformation of [pyridine-2,6-^{14}C]-labeled cetyl pyridinium chloride (monohydrate) was studied in sandy loam soil (pH 6.4, organic carbon 0.98%) from Germany (Speyer 2.3) for 121 days under aerobic conditions in darkness at 20 ± 2°C and *ca.* 40% of water holding capacity. [^{14}C]Cetyl pyridinium chloride was applied at a rate of 0.41 mg a.i./kg (equivalent to 0.46 kg a.i./ha). This study was conducted in accordance with OECD Guideline for the Testing of Chemicals, 307, Aerobic and anaerobic transformation in soil (2002); Commission Directive 95/36/EC amending Council Directive 91/414/EEC, Annex I, Section 7.1.1.1.1, Route of degradation - aerobic degradation (No. L172, 1995); Council Directive 91/414/EC concerning the placing of plant protection products on the market, Annex II, Part A, Section 7.1.1 (No. L230, 1991); and SETAC Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides (1995); and in compliance with OECD GLP Guidelines (1997). The test apparatus consisted of 1-L, cylindrical, brown metabolism flasks attached to a flow-through (humidified, CO_2-free air, flow rate not reported, purged twice daily for 30 minutes per interval) volatiles trapping system with traps for the collection of CO_2 (NaOH) and volatile organics (polyurethane foam plug, ethylene glycol monoethyl ether). Following treatment, a single flask of treated soil was taken for analysis after 0, 3, 7, 10, 14, 21, 28, 60 and 121 days of incubation. Soil was extracted three times with acetonitrile (ACN):0.5% HCl (80:20, v:v), followed by extractions with acetone and hexane, and a final 3-hour reflux extraction with ACN:0.5% HCl. The initial ambient ACN:0.5% HCl extracts were combined, concentrated via rotary evaporation (30°C), then partitioned with methylene chloride. The resulting organic phase was concentrated to dryness and the residues reconstituted in ACN for chromatographic analysis. Any processing of the acetone, hexane and reflux extracts prior to analysis was not reported. Soil extracts, extracted soil and volatile trapping materials were analyzed for total radioactivity using LSC. Soil extract samples were analyzed by reverse-phase HPLC, identifications confirmed using one-dimensional, normal-phase TLC. [^{14}C]Cetyl pyridinium chloride in soil extract samples was identified via comparison to retention times of labeled reference standard; no additional reference standards were reported.

Overall recovery of radiolabeled material averaged 102.1 ± 6.3% (range 88.0-109.9%) of the applied, with no consistent pattern of decline in total applied radioactivity. **Cetyl pyridinium chloride** dissipated in a bi-phasic pattern decreasing from 94.4% of the applied at day 0 to 51.9% at 10 days, 23.9% at 21 days, 9.3% at 60 days and was 7.8% at study termination. **Observed DT50** and **DT90** values for the parent compound occurred at 10-14 days (*ca.* 10 days) and 28-60 days (*ca.* 60 days), respectively. Dissipation was best described using first-order nonlinear

regression analysis (SigmaPlot v 9, exponential decay/single compartment, 2 parameter), with a half-life of 10 days ($r^2 = 0.9685$).

One major nonvolatile transformation product, Metabolite II, was tentatively characterized via LC/MS as a compound with a m/z value of 194.117, and a structure was proposed. No minor products were identified. **Metabolite II**, an aqueous soluble compound, was detected at a maximum 26.0% of the applied at 7 days, decreasing to 8.3-11.8% at 10-14 days and not detected thereafter. Three unidentified minor products were a total maximum 7.5% of the applied. Extractable [^{14}C]residues decreased from 98.5% of the applied at day 0 to 60.9% at 10 days, 25.5% at 21 days and were 10.3% at study termination. Nonextractable [^{14}C]residues increased to a maximum 17.8% at 10 days and were 12.4% at study termination. Mineralization to **$^{14}CO_2$** was significant totaling 80.8% of the applied at study termination, while volatile [^{14}C]organic compounds were ≤0.1% at any interval.

Results Synopsis:

Test system used: Sandy loam soil from Germany (Speyer 2.3).

Linear half-life:	34.5 days ($r^2 = 0.6386$).
Non-linear half-life:	9.8 days ($r^2 = 0.9685$).
Observed DT50:	10-14 days (*ca.* 10 days).
DT90:	

Major transformation products:	Metabolite II (m/z 194.117, structure proposed, maximum 26.0% of applied). CO_2 (maximum 80.8% of applied).
Minor transformation products:	None identified.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted in accordance with OECD Guideline for the Testing of Chemicals, 307, Aerobic and anaerobic transformation in soil (2002); Commission Directive 95/36/EC amending Council Directive 91/414/EEC, Annex I, Section 7.1.1.1.1, Route of degradation - aerobic degradation, Official Journal of the European Communities No. L172 (1995); Council Directive 91/414/EC concerning the placing of plant protection products on the market, Annex II, Part A, Section 7.1.1, Official Journal of the European Communities No. L230 (1991); and SETAC Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides (1995; p. 7). The following significant deficiencies were noted:

This study was conducted with a soil from Germany. When a foreign soil/sediment is used, the study, or an additional study, should include data from U.S.A. soils/sediments with a sufficient duration to demonstrate similarity in degradation patterns between the foreign and domestic soils/sediments regardless of microbial population differences. No soil metabolism studies in this data package include soils/sediments that would allow for a comparison between foreign and domestic soils/sediments.

Aerobic and anaerobic studies using a single soil type are typically sufficient to evaluate transformation pathways; however, rates of transformation should also be determined in at least three additional soils representing a range of relevant soil types. No additional soil metabolism studies were included in this data package to evaluate rates of transformation in differing soil types.

The major nonvolatile transformation product of cetyl pyridinium chloride, Metabolite II comprising a maximum 26.0% of the applied, was not adequately characterized. A structure for Metabolite II (m/z 194.117) was proposed based on LC/MS analyses, but identification was not confirmed against a reference standard.

Volatilized $^{14}CO_2$ was a major transformation product totaling 80.8% of the applied radioactivity at study termination; however, no supporting data were provide to confirm the identification of [^{14}C]residues recovered in the NaOH trapping solutions as $^{14}CO_2$.

No justification for selection of the test application rate was provided; consequently it could not be determined whether the test compound was applied at the maximum field use rate. Selecting a test application rate at significantly less than the maximum field use rate affects the possibility of identifying transformation products.

COMPLIANCE:

This study was conducted in compliance with OECD Good Laboratory Practice Guidelines (1997, p. 4). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4, 5, 22). A Certificate of Authenticity was not provided.

A. MATERIALS:

1. Test Material: [Pyridine-2,6-^{14}C]cetyl pyridinium chloride monohydrate (p. 9).

Chemical Structure:

Description: Technical, solid (p. 9).

Purity: Radiochemical purity: >97% via HPLC (pp. 9, 17; Figure 3 in Appendix II, p. 25).

Lot/Batch No.: 090112 (p. 9).

Analytical purity: Not reported.

Specific activity: 72 mCi/mmol (2,664 MBq/mmol, p. 9).

Location of the radiolabel: At 2- and 6-C of pyridine ring (p. 9).

Storage conditions of test chemical: Stored frozen (≤ -15°C) in darkness (p. 9).

Physical-chemical properties of cetyl pyridinium chloride monohydrate:

Parameter	Value	Comment
Molecular formula	$C_{21}H_{38}NCl(H_2O)$	
Molecular weight	358.1 g/mol	
Physical appearance	White to off-white powder.	
Water solubility	Not reported.	
Vapor pressure	Not reported.	
UV Absorption	Not reported.	
Dissociation constant (pKa)	Not reported.	
Partition coefficient (octanol/water) K_{ow}/log K_{ow}	Not reported.	
Stability of compound at room temperature	Stable at room temperature in darkness.	

Data obtained from p. 9 of the study report.

2. Soil Characteristics

Table 1: Description of soil collection and storage.

Description	Details
Geographic location	Germany, Rheinland-Pfalz, Offenbach, "Im Bildgarten", Nr. 507.
Pesticide use history at the collection site	None applied during 2004-2008.
Collection date	December 12, 2008; soil sampled by Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer, Obere Langgasse, Speyer, Germany.
Collection procedures	Not reported.
Sampling depth	0-20 cm.
Storage conditions	Soil was stored at 4°C at test facility prior to use; receipt date at test facility and storage at LUFA Speyer following collection were not reported.
Storage length	77 days; based on collection of soil on December 12, 2008, and preparation of test systems for acclimation on February 27, 2009.
Soil preparation	2-mm sieved prior to storage at test facility (p. 11). Prior to preparation of the test systems for acclimation, the soil moisture was brought to *ca.* 40% of water holding capacity using Milli-Q water; however, the specific interval this occurred was not reported.

Data obtained from pp. 10-11 of the study report.

Table 2: Properties of the soil (Speyer standard soil type 2.3).

Property	Details
Soil texture	Sandy loam.
% Sand (50-2000 µm):	60.8
% Silt (2-50 µm):	29.8
% Clay (<2 µm):	9.4

Table 2: Properties of the soil (Speyer standard soil type 2.3).

Property	Details	
pH (in $CaCl_2$)	6.4	
Organic carbon (%)	0.98	
Organic matter (%)	1.69	
CEC (meq/100 g)	8	
Moisture at 1/3 atm (%, water holding capacity)	34.4	
Bulk density, disturbed (g/cm^3)	Not reported.	
Microbial biomass (µg/g dry wt.)	Initial:	Final:
	110.9 (1.13% of OC[1])	116.0 (1.18% of OC)
Soil taxonomic classification	Not reported.	
Sol mapping unit	Not reported.	

1 Organic carbon content.
Data obtained from p. 10; Table 5 in Appendix I, p. 23 of the study report.

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: None reported.

2. Experimental conditions:

Table 3: Experimental design.

Parameter		Details
Duration of the test (posttreatment)		121 days.
Soil condition: (Air dried/fresh)		Fresh.
Soil (g/replicate)		100 g (dry/wet wt. was not specified).
Application rate (mg a.i./kg & equiv. kg a.i./ha)		0.41 mg a.i./kg dry wt. soil (0.46 kg a.i./ha[1], 0.041 mg a.i./100 g soil).
Control conditions, if used		Sterile controls were not used.
No. of Replications	Controls, if used	Sterile controls were not used.
	Treatment	Fourteen soil samples were treated with [^{14}C]cetyl pyridinium chloride monohydrate to allow for a single test system at each of nine sampling intervals, plus reserves.
Test apparatus	Type/material/volume	1-L, cylindrical, brown metabolism flasks attached to a flow-through (periodic purging) volatiles trapping system.
	Details of traps for CO_2 and volatile organics, if any	Sequentially as follows: polyurethane foam (PUF) plug (singe trap) followed by ethylene glycol monoethyl ether (single trap, *ca.* 50 mL) to

Table 3: Experimental design.

Parameter		Details
		collect organic volatiles, then 2M NaOH (two traps, each *ca.* 50 mL) to collect CO_2.
If no traps were used, is the system closed/open?		Systems were incubated closed with purging (humidified, CO_2-free air, flow rate not reported) of headspace gases twice daily for 30 minutes per interval.
Identity and concentration of co-solvent		Acetonitrile (ACN); final concentration 0.02% based on application of 0.616 mL of test solution containing 3.82% ACN to 100 g soil.
Test material application method	Volume of the test solution used/treatment:	0.616 mL.
	Application method (*e.g.*: mixed/not mixed):	Not reported.
	Is the co-solvent evaporated?	Not reported.
Any indication of the test material adsorbing to the walls of the test apparatus?		Not reported.
Microbial biomass/population of sterile controls (units)		Initial / Final
		Sterile controls were not used.
Microbial biomass/population of treated soil (units)		Initial / Final
		Not determined.
Experimental conditions:	Temperature (°C):	20 ± 2°C.
	Continuous darkness (Yes/No):	Yes.
	Moisture content:	*ca.* 40% of water holding (field) capacity.
	Moisture maintenance method:	Soil moisture was maintained at *ca.* 40% of water holding capacity throughout the incubation; however, the maintenance method was not specified.
Other details, if any		Six additional untreated nonsterile soil samples were prepared and incubated to be used for microbial biomass determinations and/or as blanks for LC/MS analyses.

1 Determined by primary reviewer using conversion rate of 1 mg a.i./kg = 1.13 kg a.i./ha.
Data obtained from pp. 6, 10-12, 16 of the study report.

3. Aerobic conditions: Prior to and following treatment, soil samples were incubated under sealed conditions with periodic purging (humidified, CO_2-free air, flow rate not reported) of headspace gases twice daily for 30 minutes per interval (p. 12). No determinations, such as redox potentials, were made to verify that aerobic conditions were maintained.

4. Supplementary experiments: None reported.

5. Sampling:

Table 4: Sampling details.

Criteria	Details
Sampling intervals for soil (days posttreatment)	0, 3, 7, 10, 14, 21, 28, 60 and 121 days.
Sampling method	A single treated soil sample was taken at each collection interval.
Method of collection of CO_2 and organic volatile compounds	Volatiles trapping materials for each respective treated soil sample were collected at each sampling interval.
Sampling intervals/times for:	
Sterility check, if sterile controls are used:	Sterile controls were not used.
Moisture content:	Not reported.
Redox potential, other:	Not reported.
Sample storage before analysis	Not reported.
Other observation, if any	None.

Data obtained from p. 12 of the study report.

C. ANALYTICAL METHODS:

Extraction/clean up/concentration methods: Soil was extracted three times with acetonitrile:0.5% HCl (80:20, v:v); extraction solvent volumes were 100 mL (p. 13). For each extraction, soil and extraction solvent were shaken (HS501 Ika-Werke platform shaker, 200 rpm) for 30 minutes; after which, extract and soil were separated via centrifugation (756 g, 5 minutes; pp. 11, 13). Extracts were combined and an aliquot (0.2 mL) analyzed for total radioactivity by LSC.

Acetonitrile was removed from the pooled sample via rotary evaporation (30°C, p. 13), with the remaining concentrate then partitioned three times with methylene chloride (3 x 100 mL). Organic phases were combined, then aliquots of the pooled organic phase (0.2 mL) and remaining aqueous phase (1 mL) were analyzed for total radioactivity by LSC. The organic phase was treated with 1% glycerol in acetone (200 µL) and concentrated via rotary evaporation (30°C), with the resulting residue reconstituted in acetonitrile (5 mL). Mean recovery of total [^{14}C]residues following the concentration step was reported as 96.3 ± 5.0% (n = 9, individual results were not provided).

Aliquots (*ca.* 50 g) of the extracted soil were then further extracted with acetone (100 mL) followed by hexane (100 mL) via shaking (platform shaker, 200 rpm) for 5 minutes per extraction; after which extract and soil were separated by centrifugation (695 g, 5 minutes; p. 13). The extracted soil was then reflux (Soxhlet) extracted with acetonitrile:0.5% HCl (80:20,

v:v, 200 mL) for 3 hours. Aliquots (0.2 mL) of each extract (acetone, hexane, acetonitrile:0.5% HCl) were analyzed for total radioactivity by LSC.

Total ^{14}C measurement: Total ^{14}C residues were determined by summing the concentrations of residues measured in the soil extracts, extracted soil and volatile trapping materials (p. 15).

Determination of non-extractable residues: Following extraction, soil samples were air dried and homogenized (method not reported), then triplicate aliquots (*ca.* 1 g) were analyzed for total radioactivity by LSC following combustion (p. 14).

Organic matter fractionation. An aliquot (*ca.* 50 g) of the 28-day extracted soil (21.3% of applied radioactivity) was further extracted with 0.1M NaOH (100 mL) via shaking (platform shaker, 200 rpm) for 30 minutes; after which, soil and extract were separated by centrifugation (2,214 g, 5 minutes; p. 13; Appendix III, p. 30). The remaining soil pellet was then washed with acetone (50 mL) via shaking (200 rpm) for 5 minutes and centrifuged (1,962 g, 5 minutes). Aliquots of the NaOH extract (1 mL) and acetone wash (0.2 mL) were analyzed for total radioactivity by LSC. The NaOH extract was acidified to pH 5 using 6M HCl and allowed to stand for 30 minutes at room temperature. The resulting precipitate (humic acids) was removed by centrifugation (2,214 g, 5 minutes). The supernatant (fulvic acids) was decanted and an aliquot (1 mL) analyzed for total radioactivity by LSC. [^{14}C]Residues recovered in the acetone wash were also considered part of the fulvic acids fraction. The humic acids pellet was re-dissolved in 0.1M NaOH (100 mL) and an aliquot (1 mL) analyzed by LSC. [^{14}C]Residues remaining in the extracted soil (humin) were analyzed by LSC following combustion.

Determination of volatile residues: Aliquots (1 mL) of the NaOH and ethylene glycol monoethyl ether trapping solutions were analyzed for total radioactivity by LSC (pp. 12-13).

Polyurethane foam (PUF) plugs were extracted twice with acetonitrile; extraction solvent volumes were 50 mL (p. 13). For each extraction, the PUF plug in the extraction solvent was tamped down ten times using a glass rod, then vortexed for 1 minute. An aliquot (1 mL) of each extract was analyzed for total radioactivity by LSC.

Derivatization method, if used: None was reported.

Identification and quantification of parent compound: Soil extract samples were analyzed by reverse-phase HPLC under the following conditions: Waters Bonda pak phenyl column (3.9 x 300 mm, 10 µm), column temperature 60°C, auto-sampler temperature 8°C, isocratic mobile phase of 30mM tetrabutylammonium bromide (TBABr) and 25mM potassium dihydrogen phosphate (KH_2PO_4) in water (pH 2.5):acetonitrile (20:80, v:v), injection volume 10 µL, flow rate 0.7 mL/minute, run time 20 minutes, UV detector (258 nm), and Lablogic β-RAM detector equipped with a 500-µL liquid cell (pp. 11, 14). Parent [^{14}C]cetyl pyridinium chloride was identified by comparison to the retention time of labeled reference standard (Table 7 in Appendix I, p. 23; Figures 5-8 in Appendix II, pp. 26-27).

Extracts (5-100 µL) were also analyzed using one-dimensional TLC on normal-phase plates (Merck; silica-60 F254) developed with n-butanol:acetic acid:Milli-Q water (40:10:50, v:v; pp. 14-15). Following development, areas of radioactivity were detected and quantified using a linear analyzer (PerkinElmer Instant Imager, p. 11). Parent [^{14}C]cetyl pyridinium chloride was identified by comparison to the R_f value of labeled reference standard (Table 7 in Appendix I, p. 23; Figures 10-11 in Appendix II, pp. 28-29).

Identification and quantification of transformation products: Transformation products were separated and quantified using HPLC and TLC as described for the parent compound (pp. 14-15; Table 7 in Appendix I, p. 23; Figures 5-8 and 10-11 in Appendix II, pp. 26-29).

Selected extracts (7 and 14 days) were analyzed using LC/MS with electrospray ionization (ESI) under the following conditions: Alltech Hypercarb LC column (2.1 x 100 mm, 5 µm), gradient mobile phase combining (A) aqueous 0.1% formic acid and (B) 0.1% formic acid in acetonitrile:Milli-Q water (90:10, v:v) [percent A:B at 0-2 min. 100:0 (v:v), 10 min. 70:30, 20-22 min. 0:100, 24-27 min. 100:0], injection volume 10 µL, flow rate 0.3 mL/minute, column temperature 21°C, LTQ Orbitrap XL MS, positive ESI, capillary temperature 300°C, photodiode array (PDA) detector (200-600 nm), and Radiomatic 525 TR radioactivity detector equipped with a 99-µL homogenous cell (Appendix V, pp. 42-43).

$^{14}CO_2$: Identification of radioactivity recovered in NaOH trapping solutions as $^{14}CO_2$ was reportedly confirmed in selected samples following precipitation with barium hydroxide; however, no additional information was provided (p. 17).

Table 5: Reference compounds available for identifying transformation products of cetyl pyridinium chloride.[1]

Applicant code	Chemical Name	Purity	Lot/Batch No.
--	--	--	--
--	--	--	--

1 None reported.

Detection limits (LOD, LOQ) for the parent compound and transformation products: Limits of detection (LOD) and quantitation (LOQ) were not reported.

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS: No supporting records were provided to establish that aerobicity, soil moisture and incubation temperature were maintained throughout the 121-day study.

B. MATERIAL BALANCE: Overall recovery of radiolabeled material averaged 102.1 ± 6.3% (range 88.0-109.9%, n = 9) of the applied, with no consistent pattern of decline in total applied radioactivity over the study duration (Table 1, p. 17).

Table 6: Biotransformation of [pyridine-2,6-^{14}C]cetyl pyridinium chloride monohydrate, expressed as percentage of applied radioactivity (n = 1), in Speyer 2.3 sandy loam soil under aerobic conditions.

Compound	Sampling times (days posttreatment)								
	0	3	7	10	14	21	28	60	121
Parent	94.4	87.9	77.9	51.9	18.4	23.9	14.8	9.3	7.8
Metabolite II	1.4	13.0	26.0	8.3	11.8	ND[2]	ND	ND	ND
Others[3]	1.4	2.9	4.9	7.5	3.6	ND	1.1	1.0	ND
Extractable residues[1]	98.5	95.5	85.8	60.9	40.0	25.5	19.8	13.3	10.3
Nonextractable residues[1]	4.7	3.6	7.4	17.8	10.6	8.8	17.4	10.7	12.4
CO_2	NP[4]	1.2	14.7	31.1	49.1	63.5	70.7	63.9	80.8
Volatile organics	NP	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1
Total recovery	103.2	100.3	108.0	109.9	99.8	97.8	107.9	88.0	103.6

1 Extractable and nonextractable residue results recalculated by primary reviewer to reflect residue results following all extractions. Data obtained from Table 1, p. 17; Table 2, p. 19; Table 6 in Appendix I, p. 23 of the study report.
2 Not detected.
3 Three unidentified [^{14}C]products (Met I, III and IV) each comprising ≤3.9% of the applied at any sampling interval (Table 8 in Appendix I, p. 24).
4 Not performed.

C. TRANSFORMATION OF PARENT COMPOUND: [^{14}C]Cetyl pyridinium chloride dissipated in a bi-phasic pattern decreasing from 94.4% of the applied at day 0 to 51.9% at 10 days, 23.9% at 21 days, 9.3% at 60 days and was 7.8% at 121 days.

HALF-LIFE/DT50/DT90: Observed DT50 and DT90 values for cetyl pyridinium chloride monohydrate in the Speyer 2.3 sandy loam soil occurred at 10-14 days (*ca.* 10 days) and 28-60 days (*ca.* 60 days), respectively. Based on first-order regression analyses (all intervals), cetyl pyridinium chloride dissipated with linear (Excel 2007) and nonlinear (SigmaPlot v 9.0) half-lives of 35 and 10 days, respectively.

Half-lives/DT50/DT90 for parent cetyl pyridinium chloride monohydrate

Phase	Half-life/DT50 (days)[1]	Regression equation	r^2	Observed DT50[2] (days)	Observed DT90[2] (days)
Speyer 2.3 sandy loam soil					
Linear/natural log	34.5	y = -0.0201x + 3.9671	0.6386	10-14 (*ca.* 10) 9.8 (actual)	28-60 (*ca.* 60) 33 (actual)
Nonlinear/normal	9.8	y = 102exp(-0.0709x)	0.9685		

1 Determined by the primary reviewer using Excel 2007 (linear, first-order) and SigmaPlot v 9.0 (nonlinear, one-compartment/two-parameter) and sample data obtained from Table 2, p. 19 of the study report.
2 Observed DT50 and DT90 values assume time 0 application as 100% to the soil.

Using single first order (SFO), first order multi-compartment (FOMC, Gustafson and Holden) and double first order in parallel (DFOP) kinetics models (ModelMaker software), the study author determined DT50 and DT90 values for cetyl pyridinium chloride of 9.7-9.8 and 32.4-32.6 days, respectively (pp. 15-16; 19-20; Figures 13-19 in Appendix IV, pp. 31-35).

TRANSFORMATION PRODUCTS: One major nonvolatile transformation product, Metabolite II, was tentatively characterized using LC/MS as a compound with a m/z value of 194.117 (p. 19; Figure 1 in Appendix V, pp. 36-48). No minor products were identified.

Metabolite II was detected in the remaining aqueous phase following organic phase partitioning of the soil extracts and increased from 1.4% of the applied at day 0 to a maximum 26.0% at 7 days, then decreased to 8.3-11.8% at 10-14 days and was not detected at 21-121 days. Using SFO kinetics, the study author determined DT50 and DT90 values for Metabolite II of 3.5 and 11.6 days, respectively (p. 20; Figure 19 in Appendix IV, p. 35).

Unidentified minor products Metabolites I, III and IV were detected at maximums of 2.2%, 3.9% and 2.3% of the applied, respectively.

NONEXTRACTABLE AND EXTRACTABLE RESIDUES: Extractable [^{14}C]residues decreased from 98.5% of the applied at day 0 to 60.9% at 10 days, 40.0% at 14 days, 25.5% at 21 days and were 10.3% at 121 days. Nonextractable [^{14}C]residues increased from 4.7% at day 0 to a maximum 17.8% at 10 days and were 12.4% at 121 days.

Organic matter fractionation of 28-day soil following the initial ACN:0.5% HCl extraction (21.3% of applied) found 2.4%, 1.3% and 17.8% of the applied associated with the fulvic acids, humic acids and humin, respectively (Table 9 in Appendix III, p. 30).

VOLATILIZATION: At 121 days, volatilized $^{14}CO_2$ totaled 80.8% of the applied radioactivity; however, results to confirm identification of $^{14}CO_2$ were not provided (Table 1, p. 17). Volatile [^{14}C]organic compounds were ≤0.1% of the applied at any interval.

TRANSFORMATION PATHWAY: A transformation pathway was not provided. Dissipation occurred through formation of one major aqueous soluble product, Metabolite II (m/z 194.117), several minor products, bound residues and extensive (>80% of applied) mineralization to CO_2.

Table 7: Chemical names and CAS numbers for the transformation products of cetyl pyridinium chloride.[1]

Applicants Code Name	CAS Number	Chemical Name	Chemical Formula	MW (g/mol)	Smiles String
--	--	--	--	--	--
--	--	--	--	--	--

1 None reported.

D. SUPPLEMENTARY EXPERIMENT-RESULTS: None reported.

III. STUDY DEFICIENCIES: The following deficiencies were noted:

- This study was conducted with a soil from Germany. USEPA recommends that studies be conducted with soils/sediments that are representative of agricultural areas where the pesticide will be used. When a foreign soil/sediment is used, the study, or an additional study, should include data from U.S.A. soils/sediments with a sufficient duration to demonstrate similarity in degradation patterns between the foreign and domestic soils/sediments regardless of microbial population differences. No additional soil metabolism studies were included in this data package that would allow for a comparison between foreign and domestic soils/sediments.

- This study was conducted with a single test soil. Aerobic and anaerobic studies using a single soil type are typically sufficient to evaluate transformation pathways; however, rates of transformation should also be determined in at least three additional soils representing a range of relevant soil types. No additional soil metabolism studies were included in this data package to evaluate rates of transformation in differing soil types.

- The major nonvolatile transformation product of cetyl pyridinium chloride, Metabolite II comprising a maximum 26.0% of the applied, was not adequately characterized. A structure for Metabolite II (m/z 194.117) was proposed based on LC/MS analyses, but identification was not confirmed against a reference standard.

- Volatilized $^{14}CO_2$ was a major transformation product totaling 80.8% of the applied radioactivity at study termination; however, no supporting data were provide to confirm the identification of [^{14}C]residues recovered in the NaOH trapping solutions as $^{14}CO_2$.

- No justification for selection of the test application rate was provided; consequently, it could not be determined whether the test compound was applied at the maximum field

use rate. Selecting a test application rate at significantly less than the maximum field use rate affects the possibility of identifying transformation products.

- Limits of detection (LOD) and quantitation (LOQ) for parent cetyl pyridinium chloride and its transformation products were not reported.

- Soil collection procedures were not described.

1. **IV. REVIEWER'S COMMENTS**

None

V. REFERENCES

1. U.S. Environmental Protection Agency. 2008. Fate, Transport and Transformation Test Guidelines, OPPTS 835.4100, Aerobic Soil Metabolism. Office of Prevention, Pesticides and Toxic Substances, Washington, DC. EPA 712-C-08-016.

2. OECD Guideline for Testing of Chemicals. 2002. Aerobic and Anaerobic Transformation in Soil. 307. Adopted by the Council on 24th April 2002.